Appendix A

LIST OF STRAINS, VECTORS, AND PLASMIDS

TABLE OF CONTENTS

A.1 Strains for Cloning and Protein Expression

DH10B

Genotype: F- *mcrA* Δ*(mrr-hsdRMS-mcrBC)* φ80d*lacZ*ΔM15 *lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara, leu*)7697 *gal*U *gal*K λ- *rps*L *nup*G *ton*A

Source: Invitrogen Max Efficiency DH10B-$T1^R$ (from Kai Yuet)

Use(s): Cloning strain. Plasmid storage and propagation. Preparation of chemically competent bacteria using the Mix & Go *E. coli* Transformation kit (Zymo Research).

Notes: Resistant to phage T1 and T5 infection (*ton*A)

Availability: (1) 25 v/v % glycerol stock

(2) Invitrogen Cat. No. 12331-013

BL21

Genotype: F- *fhuA2 [lon] ompT gal [dcm]* Δ*hsdS*

Source: New England BioLabs

Use(s): Protein expression strain. Preparation of chemically competent bacteria using the Mix & Go *E. coli* Transformation kit (Zymo Research).

Notes: Resistant to phage T1 and T5 infection (*fhuA2*, synonym for *tonA*). This strain does not contain the DE3 lysogen and cannot be used to express proteins from the T7 promoter.

Availability: (1) 25 v/v % glycerol stock

(2) NEB Cat. No. C2530H

A.2 Vectors for Cloning and Protein Expression

pUC19

Strain(s)/Plasmid: DH10B/pUC19

Antibiotic Resistance: Ampicillin

Size 2686 bp

Description: Standard cloning strain developed by Messing and coworkers (*Gene*. 33, 103-119). Site-directed mutagenesis of the P coding region to generate P variants was carried out in pUC19 due to its small size as well as the lack of elastin domains present in pQE-80L EPE, which were found to complicate the mutagenesis protocol.

The multiple cloning site of pUC19 contains numerous restriction enzymes. *EcoRI* and *XbaI* were used to subclone P.

Sequencing primers: M13F, M13R available from most sequencing facilities.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in (a) DH10B

(3) Addgene plasmid #50005

pQE-80L

Strain(s)/Plasmid: None

Antibiotic Resistance: Ampicillin

Size 4751 bp

Description: Qiagen vector for protein expression in *E coli*. T5 promoter recruits endogenous RNA polymerase (ie. DE3 lysogen is not required for T7 RNAP mediated transcription). Contains two copies of lac operator (lacO) in promoter region to regulate transcription. Contains a copy of lac repressor gene (lacI) (ie. pREP4 plasmid is not required as it is for other *trans*-repressed pQE vectors). The ribosomal binding site (RBS) is located several bp upstream of the start codon. The vector contains a sequence encoding an N-terminal 6xHis tag for IMAC purification. This can be removed by cloning at the *EcoRI* restriction enzyme site, however this site is upstream of the RBS and start codon so these elements must be included in the sequence subcloned into this site.

Note: The pQE-80L vectors used in this work were all modified to remove the XhoI restriction site at position 1.

Sequencing primers: pQEfor 5' CCC GAA AAG TGC CAC CTG
pQErev 5' GTT CTG AGG TCA TTA CTG G

Available Sources: (1) Qiagen Cat. No. 32943

A.3 Plasmids Encoding Artificial Protein Genes

pUC19 P

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pUC19 P

Vector: pUC19

Insert: P

Antibiotic Resistance: Ampicillin

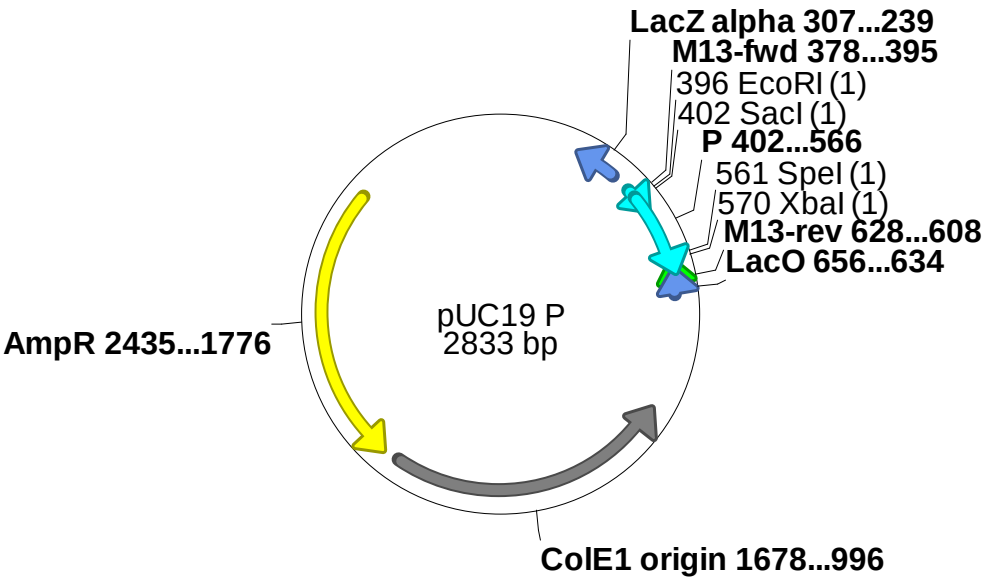
Description: P coiled coil domain in the pUC19 vector for mutagenesis. Does not contain start/stop codons. This plasmid is not for protein expression.

Construction: The P domain was amplified from pQE-80L EPE with *EcoRI* and *XbaI* overhangs, digested and subcloned into pUC19 at the same sites.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

Plasmid Map:



Coding sequence *(EcoRI to XbaI)*:

GAATTCGAGCTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAACTGCA
GGAAACCAATGCCGCGCTTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGG
AGATAACGTTCTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTA
GTGTGTCTAGA

Translation *(EcoRI to XbaI)*:

EFELGSGLGSAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASKLNTSVS
R

pUC19 A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pUC19 A

Vector: pUC19

Insert: A

Antibiotic Resistance: Ampicillin

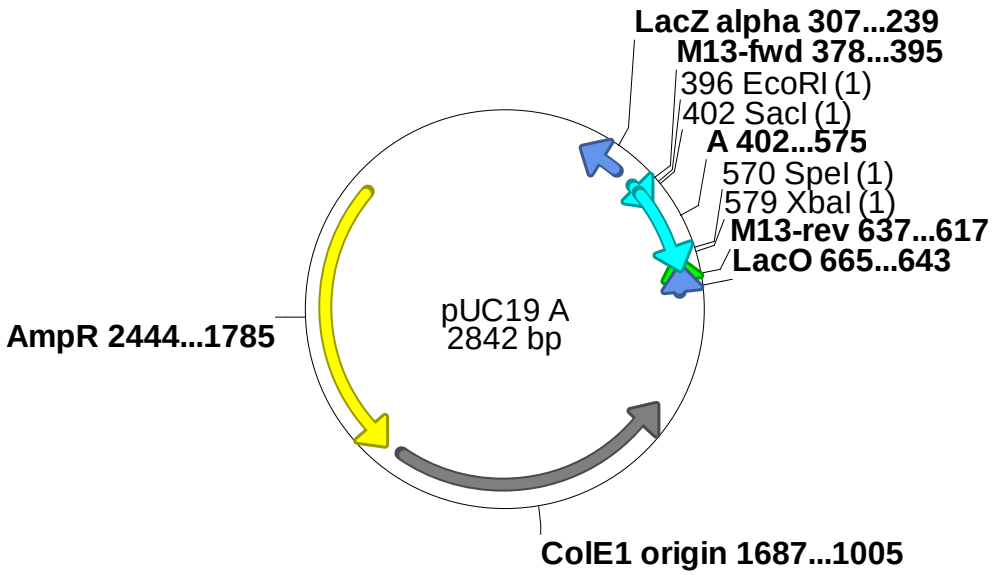
Description: A coiled coil domain in the pUC19 vector. Does not contain start/stop codons.

Construction: The A domain was amplified by PCR from pQE-9 PC10A with *SacI* and *SpeI* overhangs, digested and subcloned into pUC19 at the same sites.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

Plasmid Map:



Coding sequence *(EcoRI to XbaI)*:

GAATTCGAGCTCATGCCGACTAGCGGTGACCTGGAAAACGAAGTGGCCCAGCTGGA
AAGGGAAGTTAGATCTCTGGAAGATGAAGCGGCTGAACTGGAACAAAAAGTCTCGA
GACTGAAAAATGAAATCGAAGACCTGAAAGCCGAAATTGGTGACCATGTGGCGCCT
CGAGACACTAGTGTGTCTAGA

Translation *(EcoRI to XbaI)*:

EFELMPTSGDLENEVAQLEREVRSLEDEAAELEQKVSRLKNEIEDLKAEIGDHVAPRDTS
VSR

pUC19 P L37A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pUC19 P L37A

Vector: pUC19

Insert: P L37A

Antibiotic Resistance: Ampicillin

Description: P coiled coil domain with L37A mutation based on Gunasekar *et al.*, *Biochemistry* 2009.

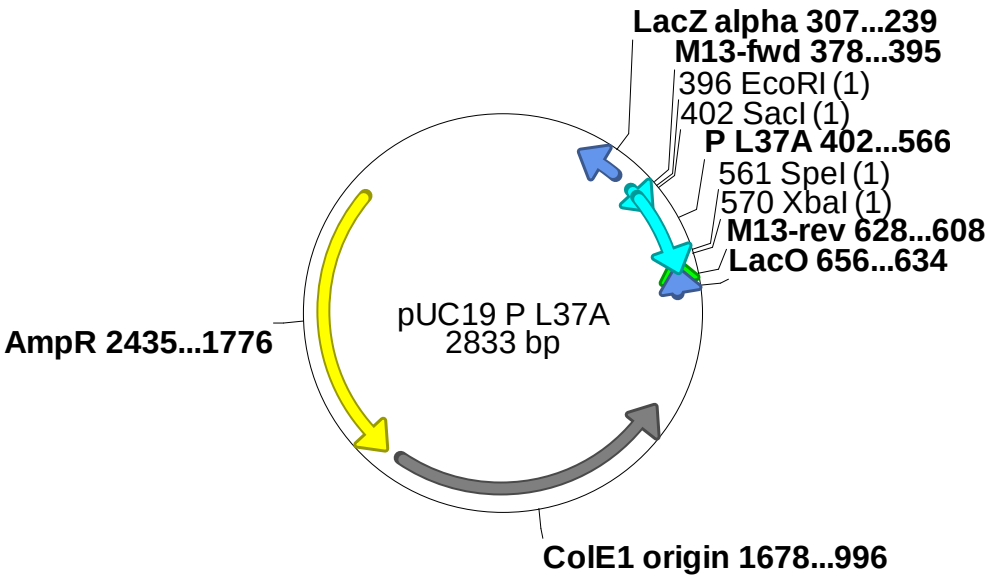
Construction: The P domain in pUC19 P was mutated by Quick Change with the following primers:

(+) CAA ATG CTG CGT GAA **GCG** CAG GAA ACC AAT GCC
(-) GGC ATT GGT TTC CTG **CGC** TTC ACG CAG CAT TTG

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

Plasmid Map:



Coding sequence *(EcoRI to XbaI)*:

GAATTCGAGCTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAAGCGCA
GGAAACCAATGCCGCGCTTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGG
AGATAACGTTCTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTA
GTGTGTCTAGA

Translation *(EcoRI to XbaI)*:

EFELGSGLGSAPQMLREAQETNAALQDVRELLRQQVKEITFLKNTVMESDASKLNTSVS
R

pUC19 P L37V

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pUC19 P L37V

Vector: pUC19

Insert: P L37V

Antibiotic Resistance: Ampicillin

Description: P coiled coil domain with L37V mutation. This mutation was not made in Gunasekar *et al.*, *Biochemistry* 2009, but is at the same position as the L37A mutation in that work.

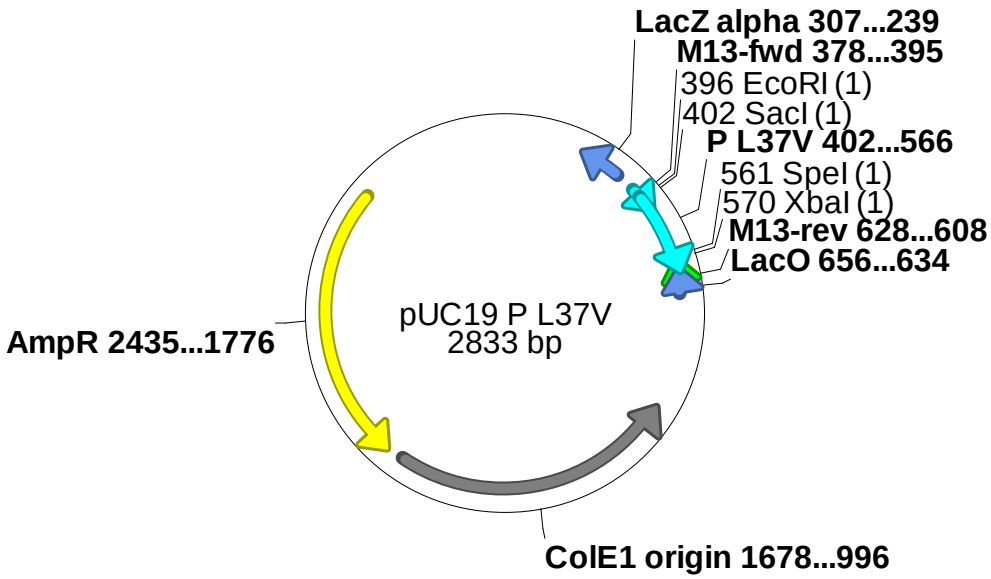
Construction: The P domain in pUC19 P was mutated by Quick Change with the following primers:

(+) CAA ATG CTG CGT GAA **GTG** CAG GAA ACC AAT GCC
(-) GGC ATT GGT TTC CTG **CAC** TTC ACG CAG CAT TTG

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

Plasmid Map:



Coding sequence *(EcoRI to XbaI)*:

GAATTCGAGCTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAAGTGCA
GGAAACCAATGCCGCGCTTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGG
AGATAACGTTCTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTA
GTGTGTCTAGA

Translation *(EcoRI to XbaI)*:

EFELGSGLGSAPQMLREVQETNAALQDVRELLRQQVKEITFLKNTVMESDASKLNTSVS
R

pUC19 P L37I

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pUC19 P L37I

Vector: pUC19

Insert: P L37I

Antibiotic Resistance: Ampicillin

Description: P coiled coil domain with L37I mutation. This mutation was not made in Gunasekar *et al.*, *Biochemistry* 2009, but is at the same position as the L37A mutation in that work.

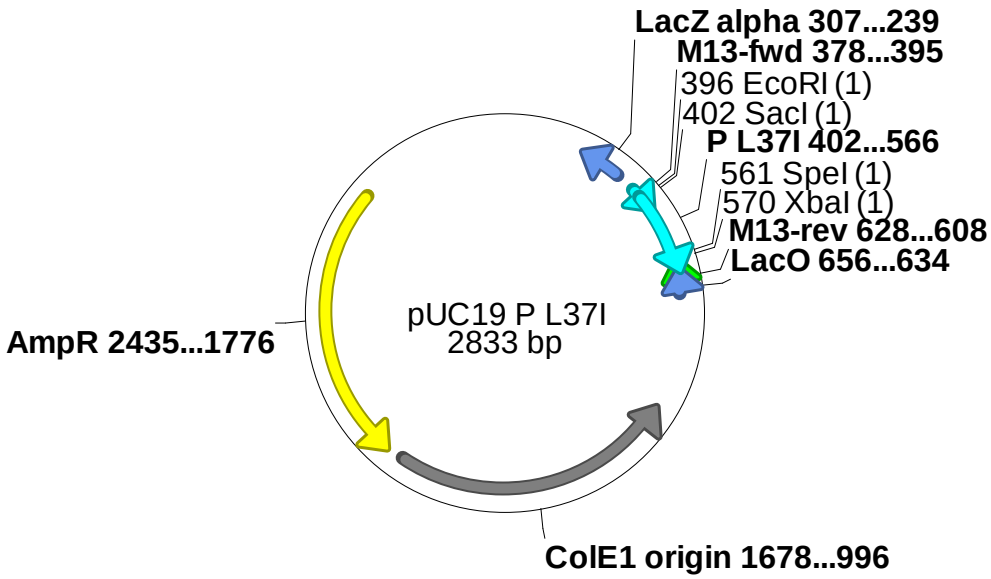
Construction: The P domain in pUC19 P was mutated by Quick Change with the following primers:

(+) CAA ATG CTG CGT GAA **ATT** CAG GAA ACC AAT GCC
(-) GGC ATT GGT TTC CTG **CAC** TTC ACG CAG CAT TTG

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

Plasmid Map:



Coding sequence *(EcoRI to XbaI)*:

GAATTCGAGCTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAAATTCA
GGAAACCAATGCCGCGCTTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGG
AGATAACGTTCTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTA
GTGTGTCTAGA

Translation *(EcoRI to XbaI)*:

EFELGSGLGSAPQMLREIQETNAALQDVRELLRQQVKEITFLKNTVMESDASKLNTSVS
R

pUC19 P L44A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B /pUC19 P L44A

Vector: pUC19

Insert: P L44A

Antibiotic Resistance: Ampicillin

Description: P coiled coil domain with L44A mutation based on Gunasekar *et al.*, *Biochemistry* 2009.

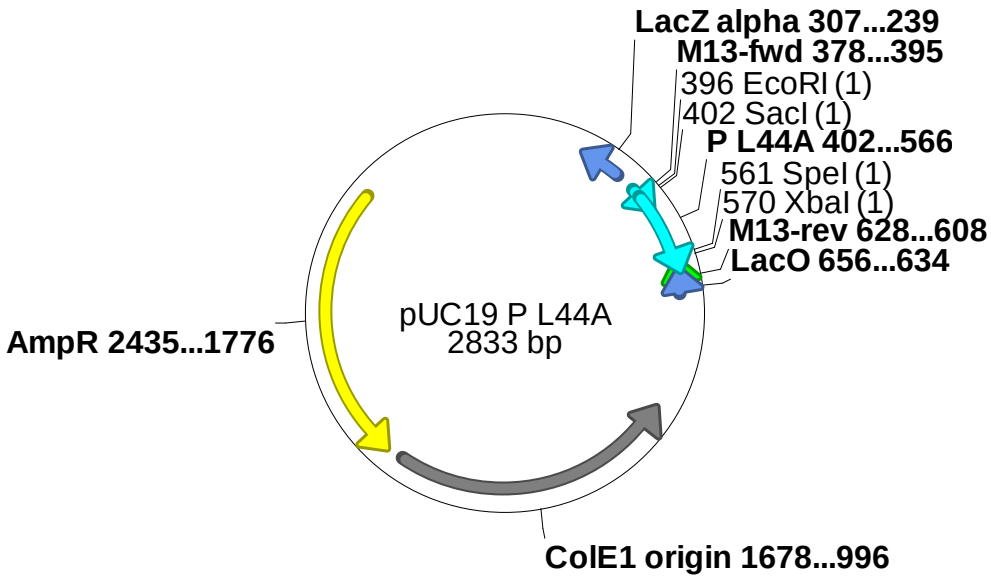
Construction: The P domain in pUC19 P was mutated by Quick Change with the following primers:

(+) CAG GAA ACC AAT GCC GCG **GCT** CAG GAT GTG CGG GAA TTG C
(-) GCA ATT CCC GCA CAT CCT GA**G** **C**CG CGG CAT TGG TTT CCT G

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

Plasmid Map:



Coding sequence *(EcoRI to XbaI)*:

GAATTCGAGCTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAACTGCA
GGAAACCAATGCCGCGGCTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGG
AGATAACGTTCTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTA
GTGTGTCTAGA

Translation *(EcoRI to XbaI)*:

EFELGSGLGSAPQMLRELQETNAAAQDVRELLRQQVKEITFLKNTVMESDASKLNTSVS
R

pUC19 P T40A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pUC19 P T40A

Vector: pUC19

Insert: P T40A

Antibiotic Resistance: Ampicillin

Description: P coiled coil domain with T40A mutation based on Gunasekar *et al.*, *Biochemistry* 2009.

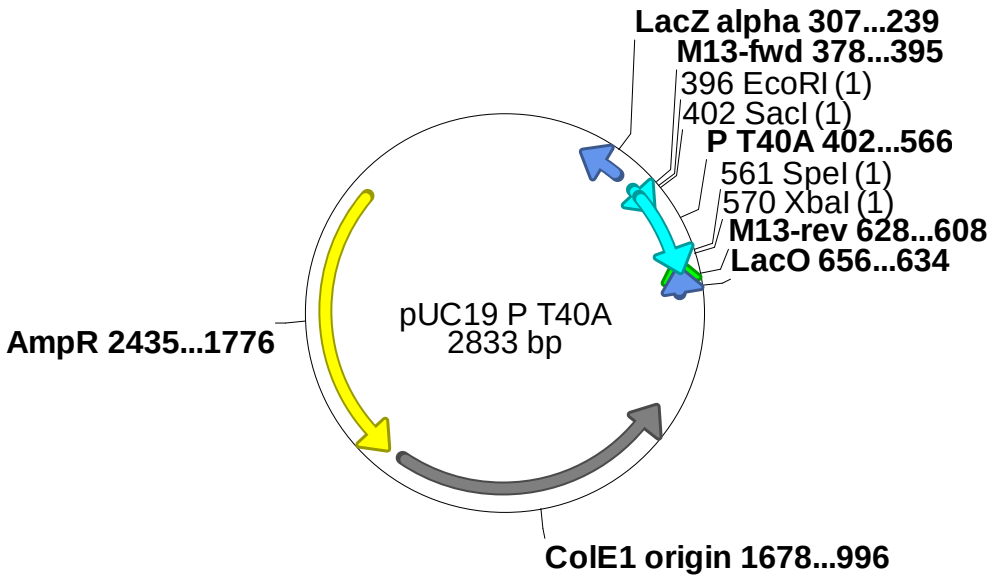
Construction: The P domain in pUC19 P was mutated by Quick Change with the following primers:

(+) GAA CTG CAG GAA **GCC** AAT GCC GCG C
(-) G CGC GGC ATT **GGC** TTC CTG CAG TTC

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

Plasmid Map:



Coding sequence *(EcoRI to XbaI)*:

GAATTCGAGCTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAACTGCA
GGAAGCCAATGCCGCGCTTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGG
AGATAACGTTCTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTA
GTGTGTCTAGA

Translation *(EcoRI to XbaI)*:

EFELGSGLGSAPQMLRELQEANAALQDVRELLRQQVKEITFLKNTVMESDASKLNTSVS
R

pUC19 P Q54A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pUC19 P Q54A

Vector: pUC19

Insert: P Q54A

Antibiotic Resistance: Ampicillin

Description: P coiled coil domain with Q54A mutation based on Gunasekar *et al., Biochemistry* 2009.

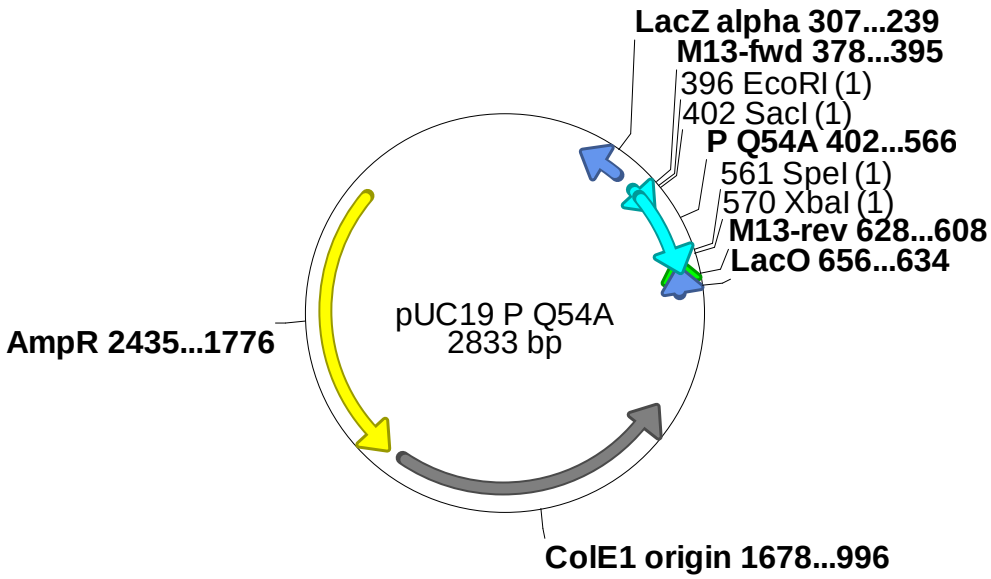
Construction: The P domain in pUC19 P was mutated by Quick Change with the following primers:

(+) GAA TTG CTT CGT CAA **GCG** GTC AAG GAG ATA AC
(-) GT TAT CTC CTT GAC **CGC** TTG ACG AAG CAA TTC

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

Plasmid Map:



Coding sequence *(EcoRI to XbaI)*:

GAATTCGAGCTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAACTGCA
GGAAACCAATGCCGCGCTTCAGGATGTGCGGGAATTGCTTCGTCAAGCGGTCAAGG
AGATAACGTTCTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTA
GTGTGTCTAGA

Translation *(EcoRI to XbaI)*:

EFELGSGLGSAPQMLRELQETNAALQDVRELLRQAVKEITFLKNTVMESDASKLNTSVS
R

pUC19 P I58A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pUC19 P I58A

Vector: pUC19

Insert: P I58A

Antibiotic Resistance: Ampicillin

Description: P coiled coil domain with I58A mutation based on Gunasekar *et al.*, *Biochemistry* 2009.

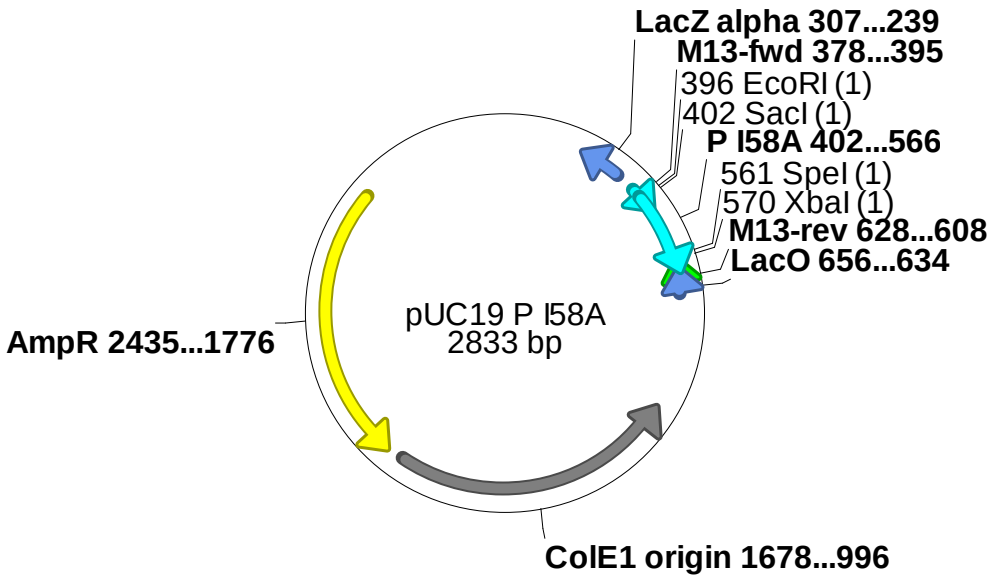
Construction: The P domain in pUC19 P was mutated by Quick Change with the following primers:

(+) CAG GTC AAG GAG **GCA** ACG TTC TTG AAG
(-) CTT CAA GAA CGT **TGC** CTC CTT GAC CTG

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

Plasmid Map:



Coding sequence *(EcoRI to XbaI)*:

GAATTCGAGCTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAACTGCA
GGAAACCAATGCCGCGCTTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGG
AGGCAACGTTCTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTA
GTGTGTCTAGA

Translation *(EcoRI to XbaI)*:

EFELGSGLGSAPQMLRELQETNAALQDVRELLRQQVKEATFLKNTVMESDASKLNTSV
SR

pQE-80L EPE

Submitted by: Wen-Bin Zhang/Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L EPE
BL21/pQE-80L EPE

Vector: pQE-80L

Insert: EPE

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a P coiled coil motif. N- and C-terminal Cys residues facilitate cross-linking with 4-arm PEG maleimide, vinyl sulfone, etc.

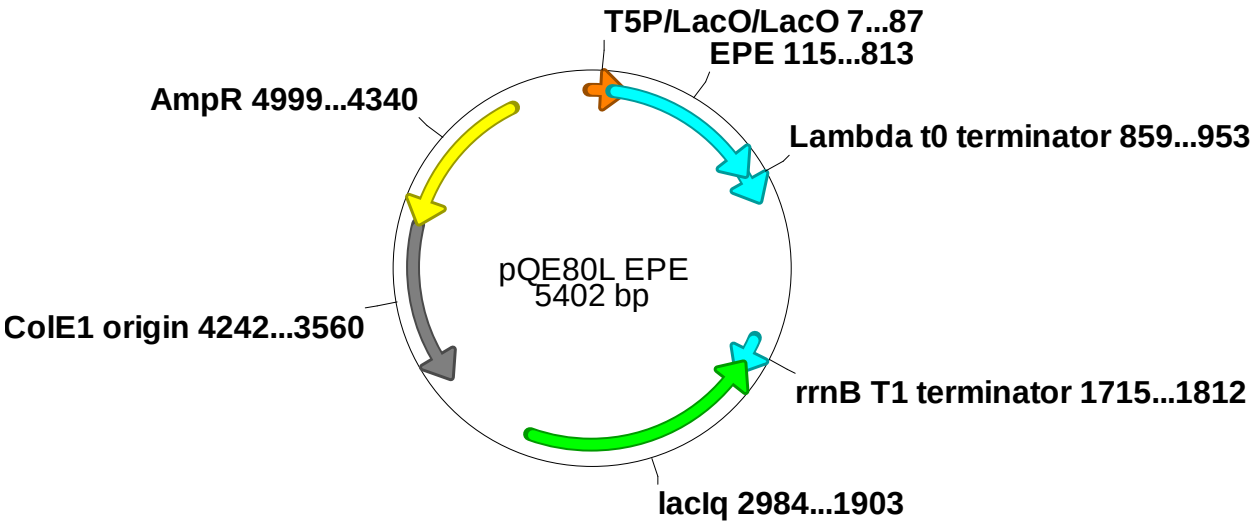
Construction: Prepared by Wen-Bin Zhang.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

ATGAGATGCAGCAGCCATCATCATCATCATCACGTCGACGGCCACGGCGTGGGTGTT
CCGGGCGTCGGTGTGCCGGGTGTGGGTGTGCCGGGCGAAGGTGTGCCGGGCGTCGG
TGTGCCGGGTGTTGGTGTTCCGGGCGTTGGTGTGCCGGGCGTTGGCGTGCCGGGCGA
GGGTGTGCCGGGCGTTGGTGTTCCGGGCGTGGGTGTGCCGGGCGTGGGCGTGCCGG
GCGTCGGTGTTCCGGGCGAGGGTGTGCCGGGCGTAGGTGTGCCGGGCGTTGGTGAG
CTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAACTGCAGGAAACCAA
TGCCGCGCTTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGGAGATAACGTT
CTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTAGTGTGCCGGG
CGTCGGCGTGCCGGGCGTAGGTGTTCCGGGCGAGGGTGTTCCGGGCGTTGGTGTGCC
GGGCGTCGGCGTGCCGGGCGTGGGTGTTCCGGGCGTAGGTGTGCCGGGCGAGGGTG
TGCCGGGCGTGGGCGTGCCGGGCGTAGGTGTTCCGGGCGTAGGTGTTCCGGGCGTA
GGTGTTCCGGGTGAAGGCGTGCCGGGCGTTGGTGTGCCGGGTGTGGGCGTGCCGGG
CGGGCTGCTCGAGTGCATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
EGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELGSGLGSAPQMLRELQETN
AALQDVRELLRQQVKEITFLKNTVMESDASKLNTSVPGVGVPGVGVPGEGVPGVGVPG
VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGG
LLECM*

pQE-80L ERE

Submitted by: Wen-Bin Zhang/Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L ERE
BL21/pQE-80L ERE

Vector: pQE-80L

Insert: ERE

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a 17mer RGD motif. N- and C-terminal Cys residues facilitate cross-linking with 4-arm PEG maleimide, vinyl sulfone, etc. The MMP1 recognition sequence (GPQGIWGQ) is included before the C-terminal Cys. Also originally denoted as CEC for Cysteine-Elastin-Cysteine.

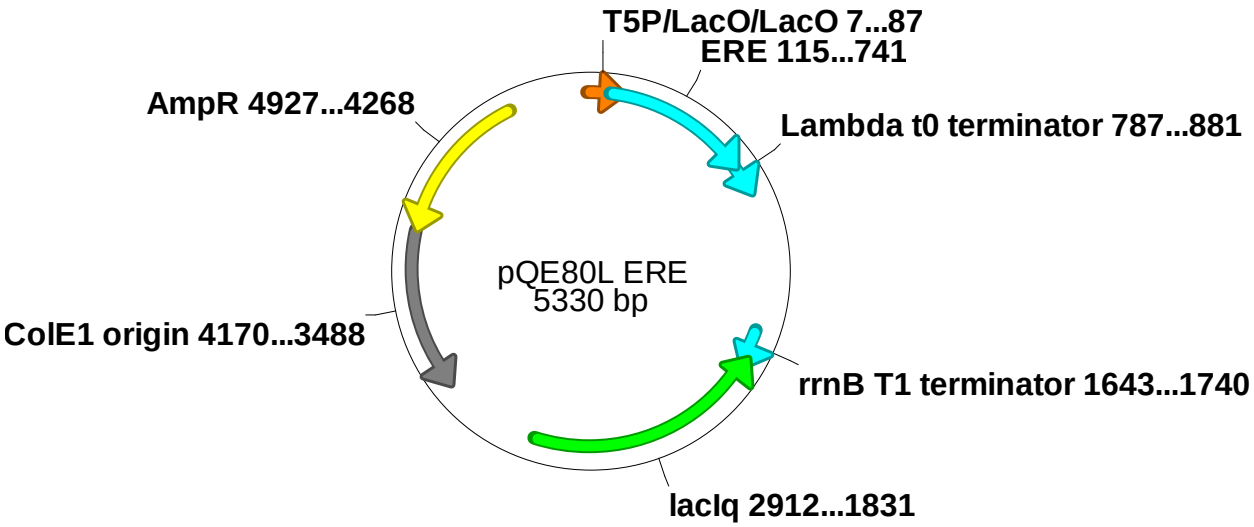
Construction: Prepared by Wen-Bin Zhang.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

ATGAGATGCAGCAGCCATCATCATCATCATCACGTCGACGGCCACGGCGTGGGTGTT
CCGGGGCGTCGGTGTGCCGGGTGTGGGTGTGCCGGGCGAAGGTGTGCCGGGCGTCGG
TGTGCCGGGTGTTGGTGTTCCGGGCGTTGGTGTGCCGGGCGTTGGCGTGCCGGGCGA
GGGTGTGCCGGGCGTTGGTGTTCCGGGCGTGGGTGTGCCGGGCGTGGGCGTGCCGG
GCGTCGGTGTTCCGGGCGAGGGTGTGCCGGGCGTAGGTGTGCCGGGCGTTGGTGAG
CTCTATGCGGTTACCGGCCGTGGTGATAGTCCGGCCAGCTCTGCCCCGATCGCCACT
AGTGTGCCGGGCGTCGGCGTGCCGGGCGTAGGTGTTCCGGGCGAGGGTGTTCCGGG
CGTTGGTGTGCCGGGCGTCGGCGTGCCGGGCGTGGGTGTTCCGGGCGTAGGTGTGCC
GGGCGAGGGTGTGCCGGGCGTGGGCGTGCCGGGCGTAGGTGTTCCGGGCGTAGGTG
TTCCGGGCGTAGGTGTTCCGGGTGAAGGCGTGCCGGGCGTTGGTGTGCCGGGTGTGG
GCGTGCCGGGCGGGCTGCTCGACGGTCCGCAAGGTATTTGGGGTCAGCTCGAGTGC
ATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
EGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELYAVTGRGDSPASSAPIATS
VPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVP
GVGVPGEGVPGVGVPGVGVPGGLLDGPQGIWGQLECM*

pQE-80L ER_CE

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L ER_CE
BL21/pQE-80L ER_CE

Vector: pQE-80L

Insert: ER_CE or ERE S104C

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a 17mer RGD motif. N- and C-terminal Cys residues facilitate cross-linking with 4-arm PEG maleimide, vinyl sulfone, etc. The MMP1 recognition sequence (GPQGIWGQ) is included before the C-terminal Cys. The RGD domains contains a Ser to Cys mutation (RGDS → RGDC) to introduce a cross-linking site in the middle of the protein. This new RGD domain is abbreviated R_C.

Construction: Serine 104 was mutated to cysteine by Quick Change of pQE-80L ERE with the following primers:

(+) GTT ACC GGC CGT GGT GAT **TGT** CCG GCC AGC TCT GCC
(-) GGC AGA GCT GGC CGG **ACA** ATC ACC ACG GCC GGT AAC

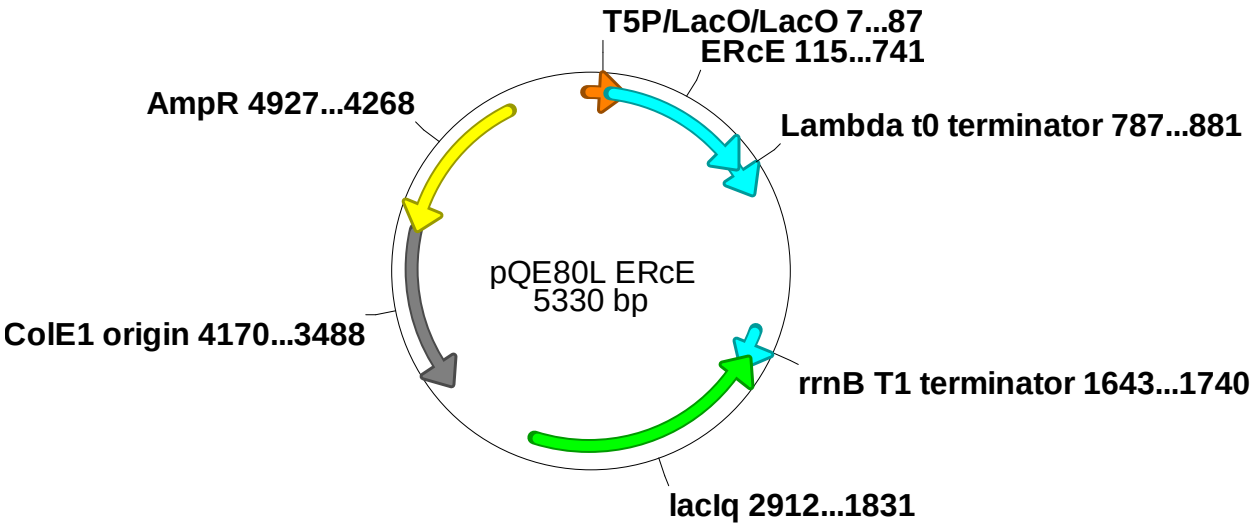
This resulted in the correct mutation but was accompanied by the deletion of the C-terminal E domain. To generate the correct construct, the *EcoRI-SpeI* fragment of this plasmid was isolated by restriction enzyme digestion and subcloned into pQE-80L ERE digested with the same enzymes (replacing ER with ER_C).

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

ATGAGATGCAGCAGCCATCATCATCATCATCACGTCGACGGCCACGGCGTGGGTGTT
CCGGGCGTCGGTGTGCCGGGTGTGGGTGTGCCGGGCGAAGGTGTGCCGGGCGTCGG
TGTGCCGGGTGTTGGTGTTCCGGGCGTTGGTGTGCCGGGCGTTGGCGTGCCGGGCGA
GGGTGTGCCGGGCGTTGGTGTTCCGGGCGTGGGTGTGCCGGGCGTGGGCGTGCCGG
GCGTCGGTGTTCCGGGCGAGGGTGTGCCGGGCGTAGGTGTGCCGGGCGTTGGTGAG
CTCTATGCGGTTACCGGCCGTGGTGATTGTCCGGCCAGCTCTGCCCCGATCGCCACT
AGTGTGCCGGGCGTCGGCGTGCCGGGCGTAGGTGTTCCGGGCGAGGGTGTTCCGGG
CGTTGGTGTGCCGGGCGTCGGCGTGCCGGGCGTGGGTGTTCCGGGCGTAGGTGTGCC
GGGCGAGGGTGTGCCGGGCGTGGGCGTGCCGGGCGTAGGTGTTCCGGGCGTAGGTG
TTCCGGGCGTAGGTGTTCCGGGTGAAGGCGTGCCGGGCGTTGGTGTGCCGGGTGTGG
GCGTGCCGGGCGGGCTGCTCGACGGTCCGCAAGGTATTTGGGGTCAGCTCGAGTGC
ATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
EGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELYAVTGRGDCPASSAPIATS
VPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVP
GVGVPGEGVPGVGVPGVGVPGGLLDGPQGIWGQLECM*

pQE-80L EAE

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L EAE
BL21/pQE-80L EAE

Vector: pQE-80L

Insert: EAE

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a A coiled coil/leucine zipper motif.

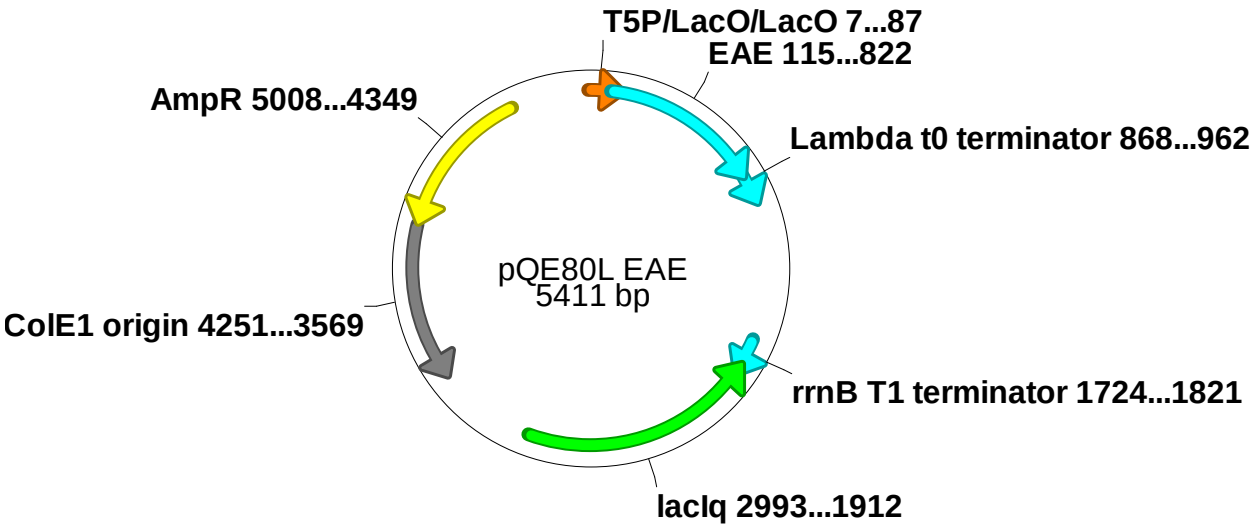
Construction: The A domain was amplified by PCR from pQE-9 PC10A with *SacI* and *SpeI* overhangs, digested and subcloned into the similarly-digested pQE-80L EPE plasmid.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

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GGGTGTGCCGGGCGTTGGTGTTCCGGGCGTGGGTGTGCCGGGCGTGGGCGTGCCGG
GCGTCGGTGTTCCGGGCGAGGGTGTGCCGGGCGTAGGTGTGCCGGGCGTTGGTGAG
CTCATGCCGACTAGCGGTGACCTGGAAAACGAAGTGGCCCAGCTGGAAAGGGAAGT
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CGTTGGTGTGCCGGGCGTCGGCGTGCCGGGCGTGGGTGTTCCGGGCGTAGGTGTGCC
GGGCGAGGGTGTGCCGGGCGTGGGCGTGCCGGGCGTAGGTGTTCCGGGCGTAGGTG
TTCCGGGCGTAGGTGTTCCGGGTGAAGGCGTGCCGGGCGTTGGTGTGCCGGGTGTGG
GCGTGCCGGGCGGGCTGCTCGAGTGCATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
EGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELMPTSGDLENEVAQLEREV
RSLEDEAAELEQKVSRLKNEIEDLKAEIGDHVAPRDTSVPGVGVPGVGVPGEGVPGVGV
PGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPG
GLLECM*

pQE-80L EPE L37A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B1/pQE-80L EPE L37A
BL21/pQE-80L EPE L37A

Vector: pQE-80L

Insert: EPE L37A

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a P coiled coil motif containing the L37A mutation.

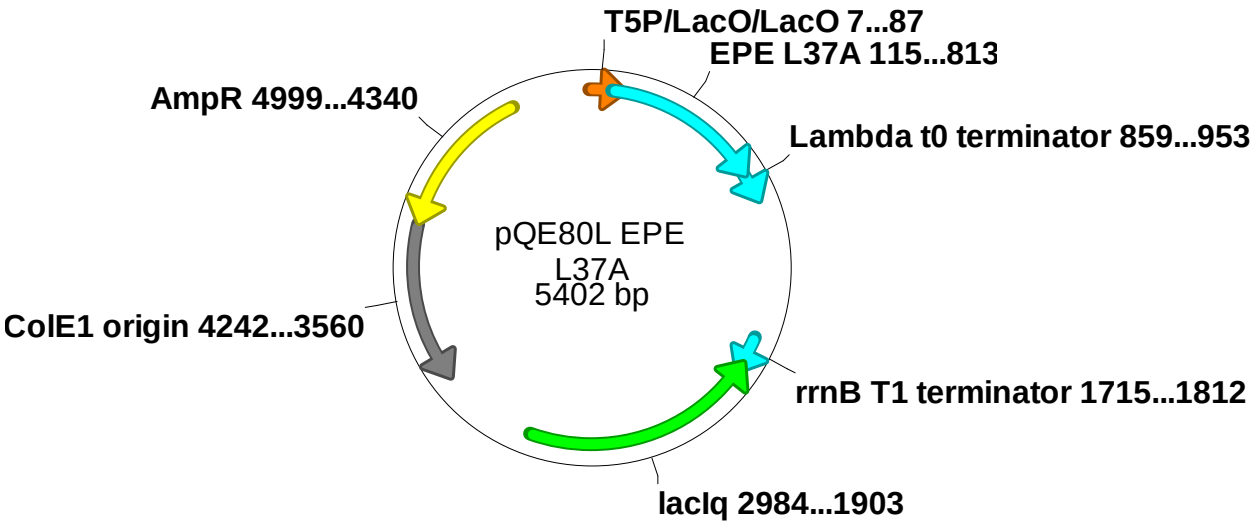
Construction: The P domain was isolated from pUC19 P L37A by digestion with *SacI* and *SpeI* and subcloned into pQE-80L EPE digested with the same enzymes.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

ATGAGATGCAGCAGCCATCATCATCATCATCACGTCGACGGCCACGGCGTGGGTGTT
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GCGTCGGTGTTCCGGGCGAGGGTGTGCCGGGCGTAGGTGTGCCGGGCGTTGGTGAG
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GGGCGTCGGCGTGCCGGGCGTGGGTGTTCCGGGCGTAGGTGTGCCGGGCGAGGGTG
TGCCGGGCGTGGGCGTGCCGGGCGTAGGTGTTCCGGGCGTAGGTGTTCCGGGCGTA
GGTGTTCCGGGTGAAGGCGTGCCGGGCGTTGGTGTGCCGGGTGTGGGCGTGCCGGG
CGGGCTGCTCGAGTGCATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
EGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELGSGLGSAPQMLREAQETN
AALQDVRELLRQQVKEITFLKNTVMESDASKLNTSVPGVGVPGVGVPGEGVPGVGVPG
VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGG
LLECM*

pQE-80L EPE L37V

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L EPE L37V
BL21/pQE-80L EPE L37V

Vector: pQE-80L

Insert: EPE L37V

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a P coiled coil motif containing the L37V mutation.

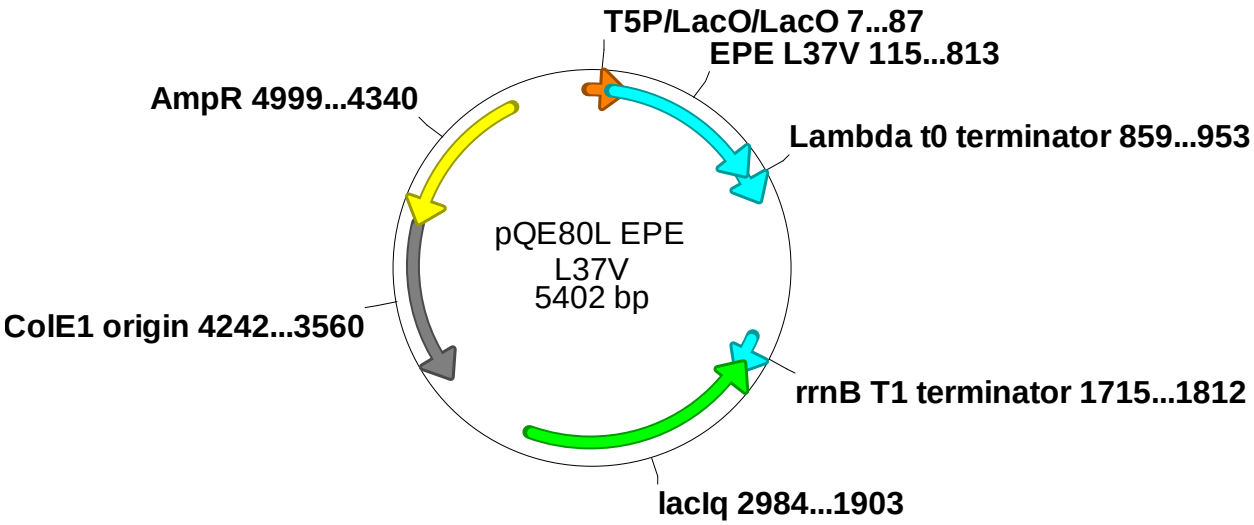
Construction: The P domain was isolated from pUC19 P L37V by digestion with *SacI* and *SpeI* and subcloned into pQE-80L EPE digested with the same enzymes.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

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CGGGCTGCTCGAGTGCATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
EGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELGSGLGSAPQMLREVQETN
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VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGG
LLECM*

pQE-80L EPE L37I

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L EPE L37I
BL21/pQE-80L EPE L37I

Vector: pQE-80L

Insert: EPE L37I

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a P coiled coil motif containing the L37I mutation.

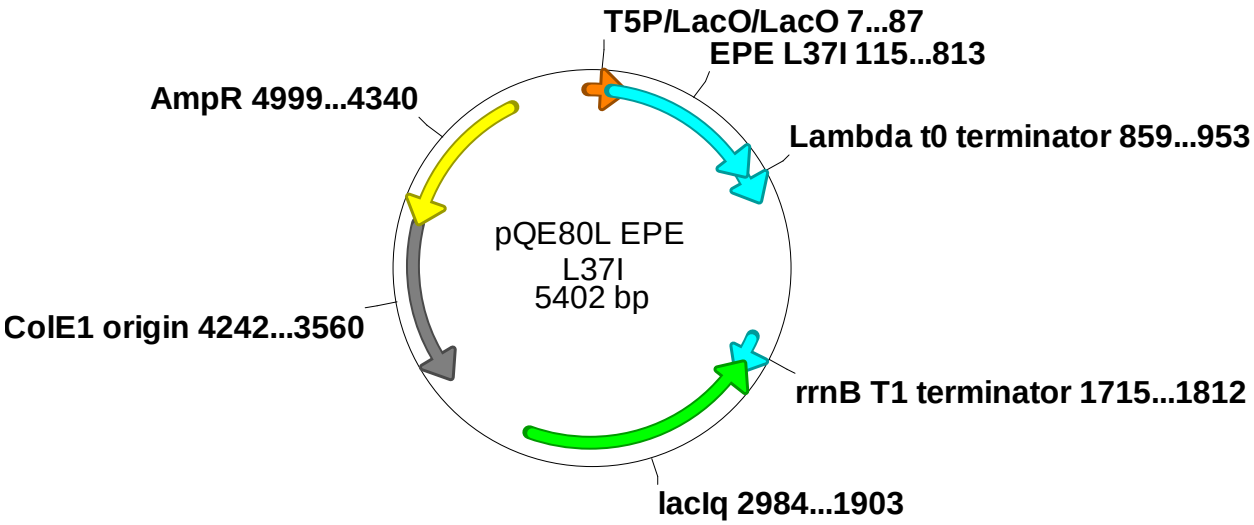
Construction: The P domain was isolated from pUC19 P L37I by digestion with *SacI* and *SpeI* and subcloned into pQE-80L EPE digested with the same enzymes.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

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GCGTCGGTGTTCCGGGCGAGGGTGTGCCGGGCGTAGGTGTGCCGGGCGTTGGTGAG
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CGGGCTGCTCGAGTGCATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
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ECM*

pQE-80L EPE L44A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L EPE L44A
BL21/pQE-80L EPE L44A

Vector: pQE-80L

Insert: EPE L44A

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a P coiled coil motif containing the L44A mutation.

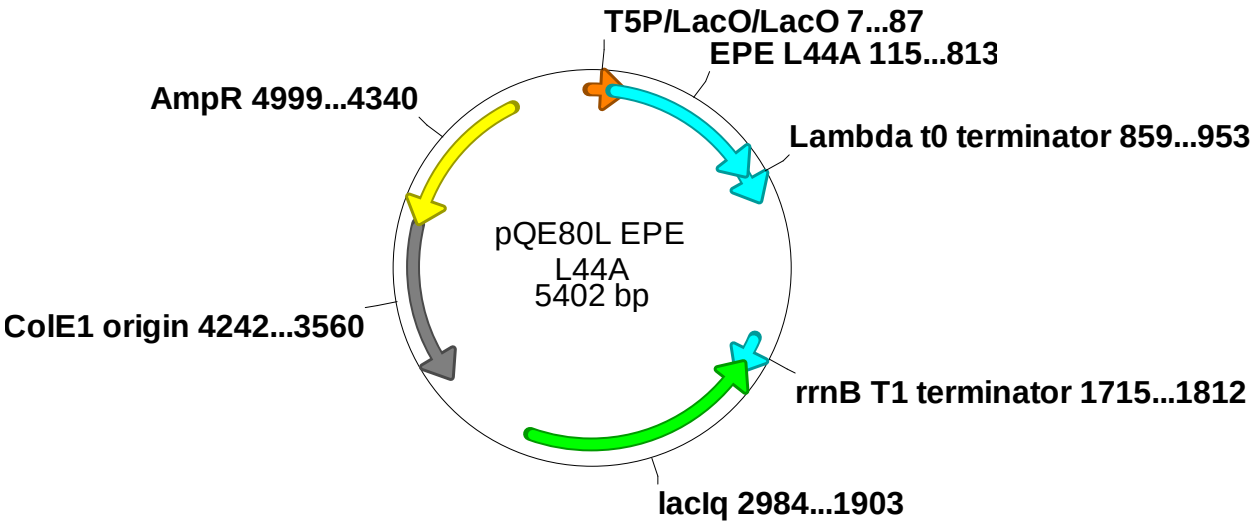
Construction: The P domain was isolated from pUC19 P L44A by digestion with *SacI* and *SpeI* and subcloned into pQE-80L EPE digested with the same enzymes.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

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TGCCGCGGCTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGGAGATAACGT
TCTTGAAGAACACCGTCATGGAGTCGGATGCGTCCAAGCTTAATACTAGTGTGCCGG
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CGGGCGTCGGCGTGCCGGGCGTGGGTGTTCCGGGCGTAGGTGTGCCGGGCGAGGGT
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GCGGGCTGCTCGAGTGCATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
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VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGG
LLECM*

pQE-80L EPE T40A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L EPE T40A
BL21/pQE-80L EPE T40A

Vector: pQE-80L

Insert: EPE T40A

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a P coiled coil motif containing the T40A mutation.

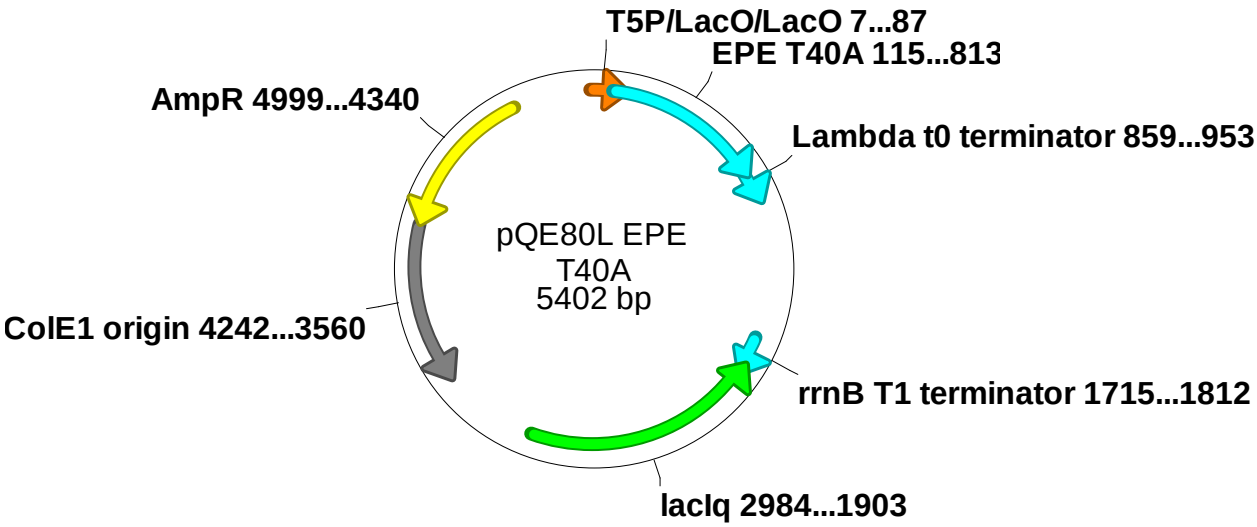
Construction: The P domain was isolated from pUC19 P T40A by digestion with *SacI* and *SpeI* and subcloned into pQE-80L EPE digested with the same enzymes.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

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GGGTGTGCCGGGCGTTGGTGTTCCGGGCGTGGGTGTGCCGGGCGTGGGCGTGCCGG
GCGTCGGTGTTCCGGGCGAGGGTGTGCCGGGCGTAGGTGTGCCGGGCGTTGGTGAG
CTCGGATCAGGACTTGGATCCGCGCCGCAAATGCTGCGTGAACTGCAGGAAGCCAA
TGCCGCGCTTCAGGATGTGCGGGAATTGCTTCGTCAACAGGTCAAGGAGATAACGTT
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CGTCGGCGTGCCGGGCGTAGGTGTTCCGGGCGAGGGTGTTCCGGGCGTTGGTGTGCC
GGGCGTCGGCGTGCCGGGCGTGGGTGTTCCGGGCGTAGGTGTGCCGGGCGAGGGTG
TGCCGGGCGTGGGCGTGCCGGGCGTAGGTGTTCCGGGCGTAGGTGTTCCGGGCGTA
GGTGTTCCGGGTGAAGGCGTGCCGGGCGTTGGTGTGCCGGGTGTGGGCGTGCCGGG
CGGGCTGCTCGAGTGCATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
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AALQDVRELLRQQVKEITFLKNTVMESDASKLNTSVPGVGVPGVGVPGEGVPGVGVPG
VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGG
LLECM*

pQE-80L EPE Q54A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L EPE Q54A
BL21/pQE-80L EPE Q54A

Vector: pQE-80L

Insert: EPE Q54A

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a P coiled coil motif containing the Q54A mutation.

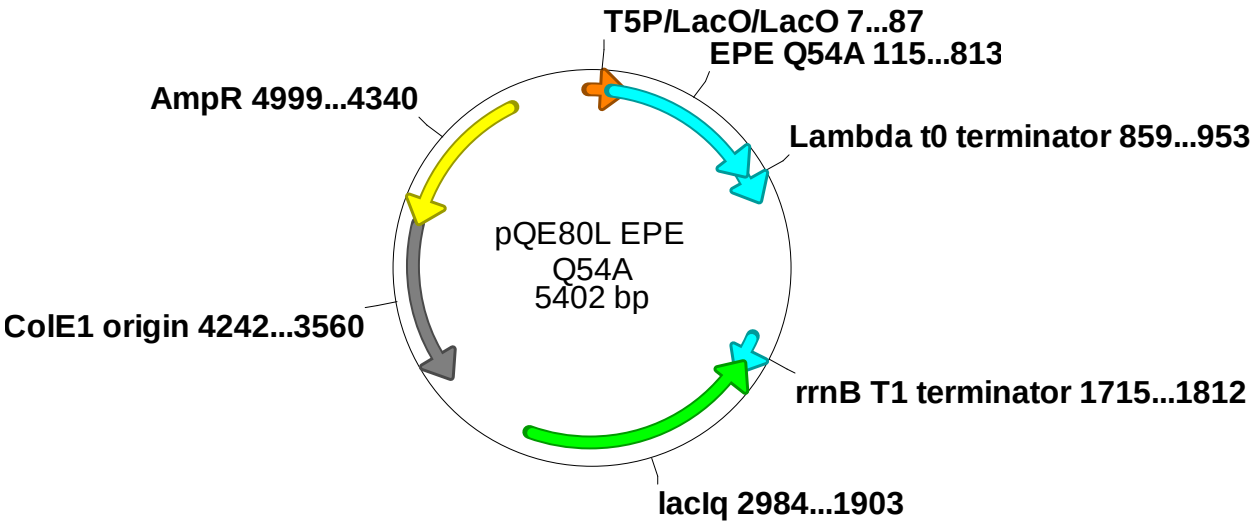
Construction: The P domain was isolated from pUC19 P Q54A by digestion with *SacI* and *SpeI* and subcloned into pQE-80L EPE digested with the same enzymes.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

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GGGTGTGCCGGGCGTTGGTGTTCCGGGCGTGGGTGTGCCGGGCGTGGGCGTGCCGG
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CGGGCTGCTCGAGTGCATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
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VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGG
LLECM*

pQE-80L EPE I58A

Submitted by: Larry Dooling

Strain(s)/Plasmid: DH10B/pQE-80L EPE I58A
BL21/pQE-80L EPE I58A

Vector: pQE-80L

Insert: EPE I58A

Antibiotic Resistance: Ampicillin

Description: Hydrophilic elastin-like repeats $[(VPGVG)_2VPGEG(VPGVG)_2]_3$ flanking a P coiled coil motif containing the I58A mutation.

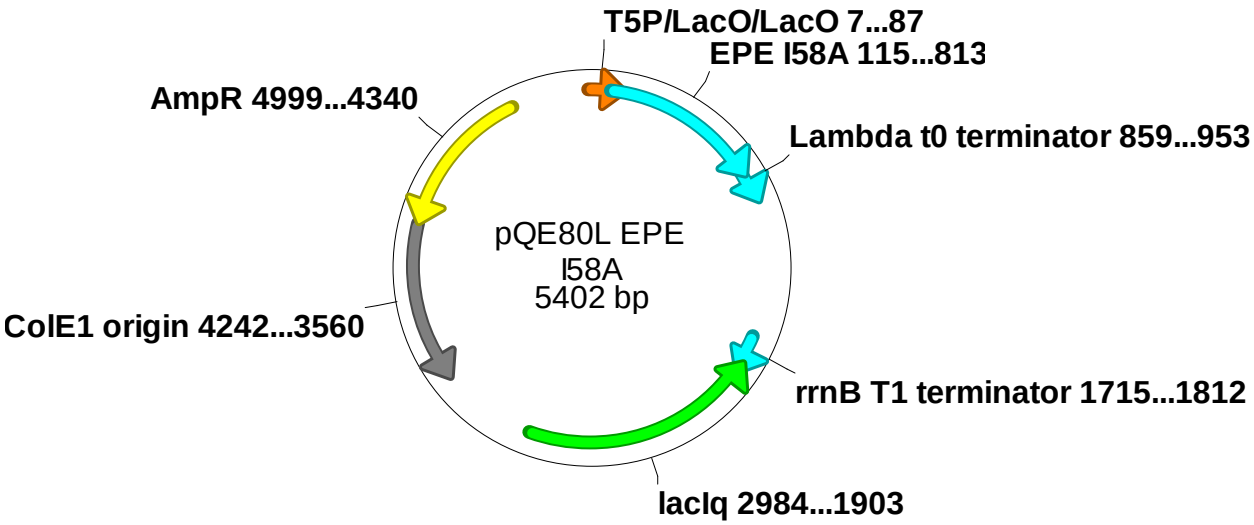
Construction: The P domain was isolated from pUC19 P I58A by digestion with *SacI* and *SpeI* and subcloned into pQE-80L EPE digested with the same enzymes.

Available Sources: (1) miniprep DNA (-20°C)

(2) 25 v/v % glycerol stock in DH10B (-80°C)

(3) 25 v/v % glycerol stock in BL21 (-80°C)

Plasmid Map:



Coding sequence:

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TGTGCCGGGTGTTGGTGTTCCGGGCGTTGGTGTGCCGGGCGTTGGCGTGCCGGGCGA
GGGTGTGCCGGGCGTTGGTGTTCCGGGCGTGGGTGTGCCGGGCGTGGGCGTGCCGG
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CGGGCTGCTCGAGTGCATGTAA

Translation:

MRCSSHHHHHHVDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPG
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GLLECM*